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c-H-*ras* (Ab-1) — Cat# OP23, OP23L

Background: The human *ras* gene family consists of three identified members, H, K and N-*ras*, encoding proteins of 188-189 amino acids and 21,000 (p21) molecular weight (1,2). Human H- and K-*ras* are the homologues of v-H- and v-K-*ras* sequences originally isolated from Harvey and Kirsten strains of rat sarcoma viruses (3,4). Normal human cellular *ras* genes can be activated to oncogenes by mutations occurring in codons 12, 13 and 61; such mutated, activated and transforming *ras* genes have been identified and isolated from human tumors and cultured tumor cells (for review see 5). Although the expression patterns of *ras* proto-oncogene proteins in normal human tissues are known (6), similar information for activated *ras* oncogene encoded p21's and their relevance to human disease diagnosis and prognosis is still emerging (7, 8, 9).

Origin: Clone F235-1.7.1 is a mouse monoclonal antibody generated by immunizing BALB/c mice with recombinant p21 protein and fusing with P3X63 Ag8.653 myeloma cells.

Characteristics:

Isotype: $IgG_1\kappa$
Epitope: within residues 54-188

Species Reactivity

human	mouse	rat	other
Y	Y	Y	NT

legend: Y=yes NT=not tested

Applications:

Immuno-Precipitation*

amount	label	positive control
5 µg per reaction	^{35}S-Met	*ras* 1 cells

Frozen Sections

amount	positive control	negative control
5 µg/mL	normal skin	*trp*E (Ab-1)

Paraffin Sections

amount	detergent	enzyme	positive control	negative control
5 µg/mL	saponin	pepsin	normal skin	*trp*E (Ab-1)

Western Blotting*

amount	chemi-luminescent	colori-metric	positive control
10 µg/mL	NT	Y	*ras* 1 cells

Immuno-fluorescence

amount	positive control
2.5 µg/mL	*ras* 1 cells

legend: Y=yes NT=not tested

*See Comments

How Supplied: 100 µg or 200 µg (Cat# OP23) of purified antibody in 1.0 mL of 0.05 M sodium phosphate buffer containing 0.1% sodium azide and 0.2% gelatin; or 100 µg (Cat# OP23L) purified antibody lyophilized from a volatile buffer with 100 µg of BSA. We recommend resuspending the lyophilized antibody with sterile phosphate buffered saline (PBS), pH 7.4, or sterile 20 mM Tris-saline (20 mM Tris containing 0.15 M NaCl), pH 7.4, to yield a final concentration of 100 µg/mL; product will be more stable if 0.1% sodium azide is included (do not add azide if antibody is to be used with viable cells). Lyophilized antibody should be resuspended at 4°C with occasional gentle mixing for at least two hours.

Storage: Store Cat# OP23 (in solution) at 4°C; do not freeze. Store Cat# OP23L (lyophilized) at 4°C until reconstituted, then store in aliquots at -20°C or at 4°C with 0.1% azide; freezing of aliquots is best for storage of reconstituted product for longer than a month, but repetitive freezing and thawing should be avoided. If stored under proper conditions, product guaranteed until expiration date stated.

Comments: For immunoprecipitation, use 5 µg Cat# OP23 per sample with 45 µL protein G plus agarose. The level of expression of $p21^{ras}$ is variable in different tissues. For this reason, we recommend a concentration step prior to western blot analysis to obtain optimal results. A doublet may be seen due to farnysylation. c-H-*ras* (Ab-1) will react to C-H-*ras* and, weakly, to v-H-*ras*; it does not detect either c-K-*ras* or c-N-*ras* p21s under the conditions tested. Purified p21 *ras* proteins are also available for western blotting standards. Suggested starting concentrations are provided. Antibodies should be titrated for optimal results in individual systems.

References:

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